Effect of Processing Temperature and Storage Time on Nonfat Dry Milk Proteins

N. PARRIS, R.A. BARFORD, A.E. WHITE, and S.M. MOZERSKY

ABSTRACT

Changes in physicochemical properties of pooled nonfat milk preheated to 63°C (I), 74°C (II), and 85°C (III), before spray-drying were examined. Insoluble material from III contained more protein (particularly at reduced pH) and more coagulated protein-lactose aggregates than either I or II. Soluble material from III was practically depleted of whey proteins which were utilized to form complexes stabilized through disulfide bonds. Milk protein micelles from III were heavier (ca 1×10^{11} g/mole) than either I or II. An unsweetened milk-orange juice blend, which was pasteurized at 63°C for 30 min and stored at 4°C, developed a precipitate which contained more protein and pectin, but less sucrose than the supernatant.

INTRODUCTION

ALTHOUGH nonfat dry milk (NFDM) is a widely used food ingredient, variation in NFDM functional properties due to preheat treatment before spray-drying limits its further utilization. High temperature preheating of NFDM used in baking is essential to improve the extensibility and water absorption of dough (Guy, 1970). However, high-heat milk powders are unsuitable for cheese making because complexes formed render the powders resistant to rennet action and coagulation (Kinsella, 1984). Effective incorporation of NFDM into high-value products such as beverages containing extracts or juices derived from fruit or vegetable sources is limited because changes occur during preheat treatment and storage. Those changes alter the ability of the powders to be rehydrated; and the functional behavior of the dispersed powders in food systems into which they are incorporated is changed. Ultra-high-temperature (UHT) pasteurization of skim milk (148°C for 3 sec) resulted in interaction of casein and whey protein, a reduction in solubility of casein and significant denaturation of whey protein in skim milk (Douglas et al., 1981). Model systems containing mixtures of soluble κ-casein and β-lactoglobulin were studied in order to explain the role of micellar dissociation (Singh and Fox, 1987), aggregation (Euber and Brunner, 1982) and hydrophobic effects (Doi et al., 1983; Haque et al., 1987; Hague and Kinsella, 1987) on the mechanism of interaction. Such model systems ignore the inhibitory effects of lactose on thermal coagulation of whey proteins (Garrett et al., 1988) and complexation of whey proteins with each other (Hillier and Lyster, 1979).

The purpose of this study was to investigate: (1) lactose and protein profiles, rehydration properties, and the size distribution of milk protein micelles for milk reconstituted from fresh and stored NFDM powders preheated at 63, 74, and 85°C before drying and at reduced pH; and (2) low-temperature stability of a milk-orange juice blend prepared from NFDM.

MATERIALS & METHODS

Sample preparation

Raw, pooled skim milk obtained from a local dairy was preheated at 63, 74, or 85°C for 30 min, cooled to 4.4°C and refrigerated overnight. The three lots were then condensed to 43% total solids at 37.8–43.3°C under 100 mm vacuum in a custom-made APV stainless steel recirculating batch vacuum evaporator. Concentrates were immediately spray-dried with a Bowen Engineering, Inc., table model laboratory spray dryer at an inlet temperature of 204.4–212.8°C and an outlet temperature of 76.7–79.7°C. Powder was cooled to 37.8°C at the collector, double-bagged and stored in metal cans at 4.4°C until used.

Solubility

Twenty grams NFDM was dispersed in 180 mL water at 25°C with stirring until all the powder was suspended in solution. Citric acid (30 mL, 0.05M) was gradually added to 70 mL of the NFDM solution (120-day samples) at 4°C to simulate that of a citrus beverage. Samples were centrifuged for 30 min (1000xg, 4°C) to remove the insolubles. The supernatants were dialyzed against water (48 hr, 4°C) to remove lactose. The retentates and insoluble pellets were lyophilized and weighed before further analysis.

Milk-orange juice blend

A milk-orange juice blend (not sweetened with succose) was prepared at 4°C and stabilized with 0.5% (w/v) pectin JM (Hercules, Wilmington, DE). The stabilized blend was carbonated and bottled, then pasteurized at 63°C for 30 min. The pH of the product was 4.2.

Protein

The lyopholized samples, ca. 3 mg, were dissolved in 1 mL Tris-urea buffer (50 mM Tris, 4 mM citrate, 5M urea, and 10 mM 2-mercaptoethanol) pH 9.0. Fifty-μl samples were quantitated by the standard dye binding protein assay (Bradford, 1976). The principal protein of milk, casein makes up 80% of the total protein. Therefore, sodium caseinate was dissolved in the same buffer and used as the protein standard. The supernatant, decanted from the milk-orange juice blend, and the remaining sediment were assayed directly using the same method.

Gel electrophoresis

Polyacrylamide gel electrophoresis of proteins in the samples was carried out on a Phast System (Pharmacia, Piscataway, NJ) using a Phast gel gradient 8-25 acrylamide. NFDM samples were prepared according to the method of Basch et al. (1985). Five mg NFDM were solubilized in 30 μL protein solvent system (0.166M Tris and 1 mM EDTA, pH 8.0), 30 μL 7% (w/v) SDS in water, and 12 μL 2-mercaptoethanol, and heated at 100°C for 5 min. After cooling, 6 μL bromophenol blue dye solution 0.1% in water was added to the sample. Mercaptoethanol was omitted in the preparation of nonreduced samples. The gels were stained with 0.2% Coomassie R350 dye and protein profiles were made with a Shimadzu Dual Wavelength TLC scanner at 550 nm.

Lactose

Lactose concentration in milk was determined by HPLC. Samples were prepared to a concentration of approximately 10 mg/mL in 0.013 N sulfuric acid. The solution was centrifuged at 25°C (7000xg, 10 min) and the supernatant was clarified by passing it through a 0.45 μ filter. Ten μL of the filtrate was injected onto an Aminex HPX-87H column (Bio-Rad, Richmond, CA); the eluant was 0.013 N sulfuric acid, with a flow rate of 0.6 ml/min; detection, RI, 16X; temperature, 23°C. The amount of lactose in the sample was determined by com-

paring its peak height to the peak height obtained from a lactose standard.

Pectin

A spectroscopic method (Blumenkrantz and Asboe-Hansen, 1973) was used to quantify pectin in the milk-orange juice blend. Sediment and supernatant samples, 0.5 mL, were diluted to 10 mL with water. Galacturonic acid was used as the standard. The concentration of pectin is expressed as grams of galacturonic acid/L supernatant or sediment.

Sucrose

Sediment and supernatant from milk-orange juice blend were adjusted to ca. pH 2 with 6N sulfuric acid. Solutions were centrifuged and clarified as described above for the analysis of lactose. Fifty μL of the filtrate was injected onto a carbohydrate column, 600 CH (Alltex Associates, Deerfield, IL); the eluant was a mixture of 85% acetonitrile and 15% water, with a flow rate of 3.5 ml/min; detector, RI, 256X; temperature 23°C.

Micelle size distribution

The size distributions of milk protein micelles reconstituted from NFDM proteins were obtained by sedimentation field flow fractionation (FFF). Ca^{+2}-free preparations of the NFDM proteins were made as described below. In some runs micelle formation was induced by adding Ca^{+2} to the protein preparation one hour prior to injection into the field flow fractionator; these are referred to as "(1-hour) preincubation" runs. In other cases, the Ca^{+2}-free protein preparation was injected, so that micelle formation took place in the fractionator when the sample was exposed to the Ca^{+2} in the FFF carrier solution; these are referred to as "no-preincubation" runs.

Low-molecular-weight solutes (including Ca^{+2}) were removed from the NFDMs by dissolving the powders in 50 mM Pipes buffer, pH 6.75, containing 160 mM KC1 and 50 mM EDTA, and by dialyzing extensively against the same solution without EDTA. For the "no-preincubation" runs, sucrose (2.0M) and water were then added to yield a sample 25 mM in Pipes buffer, 80 mM in KC1, and 300 mM in sucrose, with a protein concentration of 28 g/L. A 100-μL aliquot of this solution was injected into the fractionator.

The FFF systems utilized in this work was that previously described (Mozersky et al., 1988). The outer wall of the separation channel was lined with polyimide tape (Kapton Temp-R-Tape K-250, CHR Industries, New Haven, CT) to minimize adsorption of proteins. The FFF carrier contained 25 mM Pipes buffer, pH 6.75; 80 mM KC1; 300 mM sucrose; and 20 mM $CaC1_2$. For the "no-preincubation" runs the conditions of FFF were as follows: initial field strength (G_o), 86.2 gravities; relaxation time (t_{rlx}), 10 min; constant field time (t_c), 10 min, rotational velocity decay constant (τ_ω), 10 min; terminal (hold) field strength (G_f), 0.65 gravity; carrier flow rate (f), 1.0 mL/min. Effluent from the fractionator was monitored at a wavelength of 280 nm with a Waters Associates (Milford, MA) 450 UV absorbance detector set to a sensitivity of 0.04 AUFS. Particle weights were calculated from retention times in the usual manner (Mozersky et al., 1988).

For the "(1-hr) preincubation" runs, sample preparation was as above except that $CaCl_2$ was added after sucrose to provide a final $CaCl_2$ concentration of 20 mM. Except for the presence of protein, the composition of samples preincubated with Ca^{+2} was therefore the same as that of the FFF carrier (given above). The sample protein concentration was 7.0 g/L. A 327-μL aliquot of this solution was injected into the fractionator. The conditions of FFF were: G_o, 5.5 xg; t_{rlx}), 10 min; t_c, 10 min; τ_ω, 10 min; G_f, 0.16 xg; f, 1.0 mL/min. Effluent was monitored as described above.

RESULTS & DISCUSSION

SOLUBILITY CHARACTERISTICS of rehydrated NFDM powders are shown in Table 1 as percent insoluble protein. More insoluble protein was found in NFDM subjected to high preheat treatment (85°C), particularly at reduced pH, than in the other portions. Douglas et al. (1981) reported reduced solubility for UHT pasteruized milk (148°C for 3 sec) at pH 6.0. There was no apparent increase in insoluble protein with storage time however, we can not at this time explain the higher insoluble protein values after 120 days compared to 240 days of storage. Insolubles from the powders subjected to high preheat treatment contained more lactose (Table 2). Although the disaccharides sucrose and lactose have been reported to inhibit thermal coagulation of unfractionated whey proteins (Garrett et al., 1988), coagulation does occur in the NFDM subjected to higher preheat temperatures. It appears that lactose interacts with protein to form larger aggregates which coagulate. The amount of coagulation increased with increasing preheat temperature and storage time of the NFDM as indicated by the lower protein/lactose values (Table 2).

Table 1—Percent insoluble protein in reconstituted nonfat dry milk (%)

	63°C[a]	74°C[a]	85°C[a]
10 Day	0.98	0.68	1.38
120 Day	1.00	1.15	2.18
240 Day	0.64	0.76	1.74
pH 6.0	1.36	1.09	3.50

[a] Preheat treatment temperatures of skim milk before spray drying.

Table 2—Effect of preheat treatment on % protein/% lactose ratio

	63°C[a]	74°C[a]	85°C[a]
Powder	0.76	0.79	0.67
Insoluble[b] - 10 day	3.70	3.26	1.67
Insoluble[b] - 120 day	2.80	2.43	1.87
Insoluble[b] - 240 day	2.18	2.10	1.55
Insoluble[b] - pH 6.0	3.44	3.29	1.94

[a] Preheat temperatures.
[b] Isolated by centrifugation (1000 xg, 4°C).

Table 3—Milk components in soluble portion of rehydrated nonfat dry milk[a] (%)

Proteins[b]	63°C[c]	74°C[c]	85°C[c]
α_s - Caseins	34.5	37.7	41.8
β-, κ - Caseins	24.5	27.3	31.0
β - Lactoglobulin (β-Lg)	19.0	14.7	2.8
α - Lactalbumin (α-La)	4.6	4.7	0.5

[a] From SDS-PAGE.
[b] Samples made up to the same concentration.
[c] Preheat temperatures.

Gel electrophoresis (SDS-PAGE) of the insoluble material from the high-preheat-treated (85°C) NFDM powders (not shown) indicated the presence of material of very high molecular weight that did not enter the running gel. This material probably resulted from coagulation of protein and lactose. These insolubles were not found in the medium and low-preheat-treated NFDM powders. In addition, SDS-PAGE of dialyzed solubles for the high-heat-treated NFDM powders showed that whey proteins, β-lactoglobulin and α-lactalbumin are practically depleted from the NFDM and that the amount of caseins increased with increasing preheat treatment temperature (Table 3). It appears that the whey proteins are being utilized to form new compounds which migrate with the caseins as demonstrated by an increase in the intensity of the casein bands. Densitometric scans of gels of nonreduced protein from the soluble fraction of 63°C and 85°C preheat NFDM powders (Fig. 1A and Fig. 1B) showed that bovine serum albumin (BSA) was also utilized in the formation of these new compounds. This confirms earlier work using kinetic analysis of whey denaturation (Hillier and Lyster, 1979) that between 40 and 100°C BSA was involved in the overall complex formation. These new compounds were apparently stabilized through disulfide linkage, since they were no longer present in reduced gels of solubles from 85°C preheat NFDM powder and the whey proteins were again present (Fig. 1C).

Caseins in milk exist as large colloidal Ca^{+2}-containing complexes known as micelles. In the absence of Ca^{+2}, they exist as much smaller protein complexes referred to as submicelles. In order to focus on the influence of processing temperature on the ability of the protein components of NFDM to form protein micelles, we observed micelle formation after the

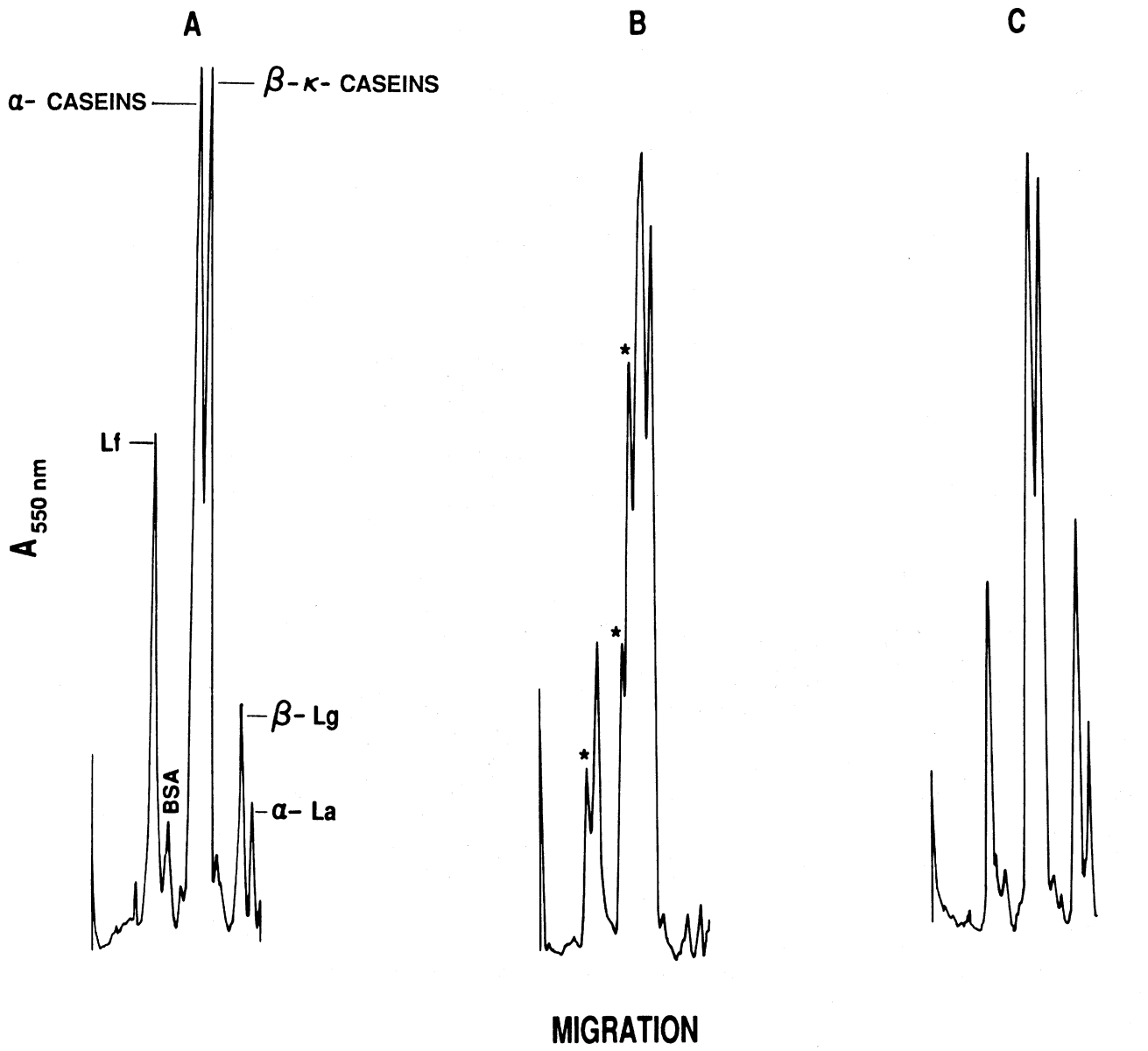

Fig. 1. – Densitometry profiles of SDS-PAGE gels for soluble protein (non-reduced), (A) 63°C, (B)85°C and (reduced), (C) 85°C. (*) new compounds formed.

addition of Ca^{+2} to Ca^{+2}-free (submicellar) solutions of the NFDM proteins prepared as described (Materials and Methods).

To permit observation of micelle formation within 1/2 hr after addition of Ca^{+2}, the Ca^{+2}-free NFDM protein solutions were injected into a flowing stream of FFF carrier containing Ca^{+2} ("no-preincubation" runs). After the sample had been introduced into the separation channel of the fractionator, relaxation and elution proceeded as usual. As the processing temperature increased from 63 to 85°C, the retained particles observed (peak 2) increased in size and amount (Fig 2). The average particle size was estimated from retention times of 15.5, 22, and 37.5 min to be 2×10^9 at 63°C, 6×10^9 at 74°C, and 1×10^{11} g/mole at 85°C. When Ca^{2+}-containing carrier was replaced with Ca^{+2}-free carrier (not shown), essentially all of the protein eluted in peak 1, with little or no difference among the three NFDM samples.

When the samples were preincubated in the medium containing Ca^{+2}, a component, which was very strongly retained in the fractinator channel, even at very low field strength, was observed (not shown) for the NFDM processed at 85°C. The apparent particle weight was $>> 10^{11}$ g/mole. This component was absent from the NFDM processed at lower temperature. This result is consistent with the "no-preincubation" result. In both cases protein micelles of larger size were seen in the high-temperature processed NFDM.

A milk-orange juice blend was prepard as described. Sediment precipitated from the blend on standing at 4°C. The stabilizer did not prevent protein precipitation since the sediment contained 3 times as much protein as the supernatant (Table 4). A higher concentration of pectin (expressed as glacturonic acid) was found in the sediment. Pectin may be complexed with protein in the sediment. The protein constituent of commercial lemon juice cloud was shown to be protein complexed with material from the albedo of the fruit which is rich in pectin (Klavons and Bennett, 1985). As expected, a most of the sucrose from the orange juice was found in the supernatant.

The protein solubility of milk powders may be improved by redispersing the powder at an elevated pH and readjusting the pH to the desired level as has been reported by Douglas et al. (1981). Although the milk-orange juice blend was not completely stable, other stabilizers may more effectively stabilize

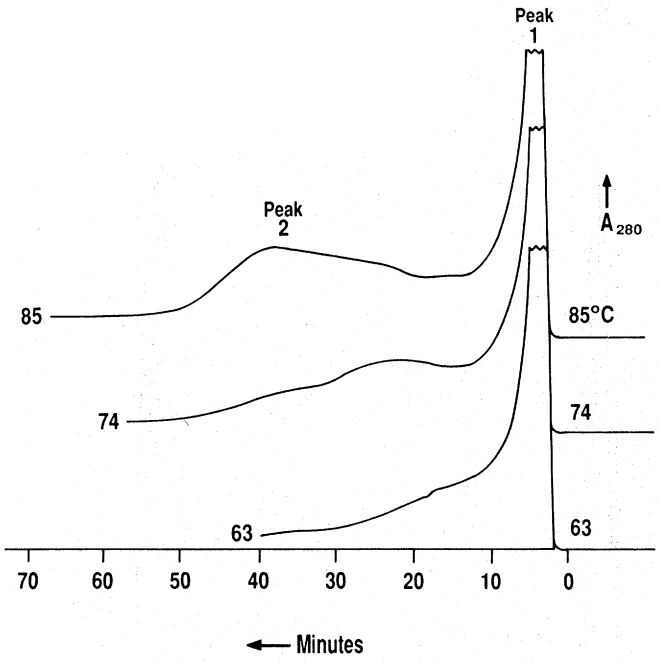

Fig. 2.—Fractograms of absorbance at 280 nm (A_{280}) obtained on field flow fractionation of protein micelle preparations from NFDM powders preheated to 63, 74, and 85°C. In each case, a Ca^{+2}-free solution of the NFDM protein was injected into a carrier containing 20 mM Ca^{+2}. Conditions are given in the text.

Table 4—Analysis of milk-orange juice blend

Component	Sediment (g/L)	Supernatant (g/L)
Protein	10.6	3.6
Galacturonic acid	8.4	6.4
Sucrose	18.3	48.9

the suspension when used alone or in combination. They can not, however, affect other functional properties, e.g., viscosity of the product, to be of value. Such information should delineate strategies for improving the functional properties of NFDM and thereby increase its utilization of new foods.

In conclusion, low-heat NFDM is more suitable for use in milk-orange juice beverages, since less insoluble protein and less protein-lactose coagulation occurs with that type of NFDM. At recuded pH, however, more insoluble protein is formed which adversely affects the mouthfeel of the product.

REFERENCES

Basch, J.J., Douglas, F.W., Jr., Procino, L.G., Holsinger, V.H., and Farrell, H.M., Jr. 1985. Quantitation of caseins and whey proteins of processed milks and whey protein concentrates. Application of gel electrophoresis, and comparison with Harland-Ashworth procedure. J. Dairy Sci. 68: 23.

Blumenkrantz, W. and Asboe-Hansen, G. 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54: 484.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein dye binding. Anal. Biochem. 72: 248.

Doi, H., Ideno, S., Ibuki, F., and Kanamori, M. 1983. Participation of the hydrophobic bond in complex formation between κ-casein and β-lactoglobulin. Agric. Biol. Chem. 47: 407.

Douglas, F.W., Jr., Greenberg, R. Farrell, H.M. Jr., and Edmonson, L.F. 1981. Effects of ultra-high-temperature pasteurization on milk proteins. J. Agric. Food Chem. 29: 11.

Euber, J.R. and Brunner, J.R. 1982. Interactions of κ-casein with immobilized β-lactoglobulin. J. Dairy Sci. 65: 2384.

Garrett, J.M., Stairs, R.A., and Annett, R.G. 1988. Thermal denaturation and coagulation of whey proteins: Effects of sugars. J. Dairy Sci. 71: 10.

Guy, E.J. 1970. In "By-Products from Milk," B.H. Webb and E.O. Whittier (Ed.), p. 197. AVI Publishing Co., Westport, CT.

Hague, Z. and Kinsella, J.E. 1988. Interaction between heated κ-casein and β-lactoglobulin: predominance of hydrophobic interactions in the initial stages of complex formation. J. Dairy Res. 55: 67.

Haque, A., Kristjansson, M.M., and Kinsella, J.E. 1987. Interaction between κ-casein and β-lactoglobulin: Possible mechanism. J. Agric. Food Chem. 35: 644.

Hillier, R.M. and Lyster, R.L.J. 1979. Whey protein denaturation in heated milk and cheese whey. J. Dairy Res. 46: 95.

Kinsella, J.E. 1984. Milk proteins: Physicochemical and functional properties.Critical Reviews Food Sci. & Nutr. 21: 197.

Klavons, J.A. and Bennett, R.D. 1985. The nature of the protein constituent of commercial lemon juice cloud. J. Agric. Food Chem. 33: 70.

Mozersky, S.M., Caldwell, K.D. Jones, S.B., Maleeff, B.E., and Barford, R.A. 1988. Sedimentation field flow fractionation of mitochondrial and microsomal membranes from corn roots. Anal. Biochem. 172: 113.

Singh, H. and Fox, P.F. 1987. Heat stability of milk: role of β-lactoglogulin in the pH-dependent dissociation of micellar κ-casein. J. Dairy Res. 54: 509.

Ms received 10/11/88;, revised 2/21/89; accepted 2/25/89.